Viral nucleic acid isolation

User manual

NucleoSpin® Dx Virus

CE

IVD *In-Vitro* Diagnostic Medical Device

REF 740895.50

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July 2014 / Rev. 04

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1 Components

1.1 Kit contents

		NucleoSpin® Dx Virus
		50 preps
REF	Symbol	740895.50
Lysis Buffer RAV1	BUF RAV1	35 mL
Wash Buffer RAW	BUF RAW	30 mL
Wash Buffer RAV3 (Concentrate)*	BUF RAV3 Conc.	12 mL
RNase-free H_2O	H_2O	13 mL
Elution Buffer RE**	BUF RE	13 mL
Carrier RNA (lyophilized)	Carrier RNA	1 mg
Proteinase Buffer PB	BUF PB	1.8 mL
Proteinase K (lyophilized)*	Proteinase K	30 mg
NucleoSpin® Dx Virus Columns (dark blue rings - plus Collection Tubes)	Dx Virus Columns	50
Collection Tubes (2 mL)	Collection Tubes	4 x 50
Lysis Tubes (1.5 mL)	Lysis Tubes	50
Elution Tubes (1.5 mL)	Elution Tubes	50
User manual		1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer RE: 5 mM Tris/HCl, pH 8.5

EN	Do not reuse	Use by	Batch identification	Item number	Contains sufficient for <n> tests	Manufacturer	*In vitro* diagnostic products	Please read instructions for use!	Permitted storage-temperature range
DE	Nicht wiederverwenden	Verwendbar bis	Chargencode	Artikelnummer	Ausreichend für <n> Prüfungen	Hersteller	*In-vitro*-Diagnostikum	Gebrauchsanweisung beachten	Temperaturbegrenzung
ES	Producto de un solo uso	Fecha de caducidad	Código de lote	Referencia	Contenido suficiente para <n> tests	Fabricante	Diagnóstico *in vitro*	Obsérvense las instrucciones de uso	Límites de temperatura
IT	Non riutilizzare	Utilizzare entro	Codice del lotto	Numero di catalogo	Contenuto sufficiente per <n> test	Fabbricante	Dispositivo medico-diagnostico in vitro	Consultare le istruzioni per l'uso	Limiti di temperatura
FR	Ne pas réutiliser	À utiliser avant	Numéro de lot	Référence	Contenu suffisant pour "n" tests	Fabricant	Diagnostic *in vitro*	Respecter les instructions d'utilisation	Limites de température
NL	Niet hergebruiken	Te gebruiken tot	Productienummer	Artikelnummer	Voldoende voor <n> tests	Fabrikant	In-vitro diagnosticum	Lees de bijsluiter	Temperatuur begrenzing
DA	Må ikke bruges igen	Holdbar til	Batch kode	Artikelnummer	Tilsttrækkeli gt til <n< kontroller	Producent	*in-vitro*-diagnostikum	Følg brugsanvisningen	Temperaturbegrænsning
EL	Προϊόν μιας χρήσης	Ημερομηνία λήξης	Αρ. παρτίδας	Αρ. είδους	Επαρκεί για <n> δοκιμές	Κατασκευαστής	Διαγνωστικό in vitro	Τηρείτε τις οδηγίες χρήσης	Όρια θερμοκρασί ας
PL	Nie używać ponownie	Przydatno ść do użycia	Numer partii	Numer artykułu	Wystarcza jąco dla kontroli <n>	Producent	Diagnosty ka in-vitro	Przestrzeg ać instrukcję użycia	Ogranicze nie temperatur y
PT	Não reutilizar	Data de vencime	Número de lote	Número de artigo	Suficiente para <n> testes	Fabricante	Diagnóstico in vitro	Observar as instruções de uso	Limites de temperatura
SV	Återanvänd ej	Använd för	Lot nummer	Katalognummer	Räcker till „n" antal tester	Tillverkare	Medicintekniska produkter för in vitro diagnostik	Se handhavandebeskrivningen	Temperaturbegränsning

EN	Irritant	Harmful	Flammable
DE	Reizend	Gesundheitsschädlich	Entzündbar
ES	Irritante	Nocivo	Inflamable
IT	Irritant	Nocivo	Infiammabile
FR	Irritante	Nocif	Inflammable
NL	Irritierend	Schadelijk	Ontvlambaar
DA	Lokalirriterende	Skadelig	Brandfarlig
EL	Διαβρω-τικό	Επιβλαβής	Εύφλεκτα
PT	Irritante	Nocivo	Inflamável
SV	Irritanderende	Skadligt	Brandfarliga

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol (to adjust nucleic acid binding conditions and to prepare Wash Buffer RAV3)

Consumables

- Disposable pipet tips (aerosol barrier pipet tips are recommended to avoid cross-contamination)

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Vortex mixer
- Heating block or water bath for 70 °C incubation
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended to read the detailed protocol section of this user manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

MACHEREY-NAGEL user manuals are available on the internet at ***www.mn-net.com***.

2 Product description

2.1 Intended use

The **NucleoSpin® Dx Virus** kit is a generic system for the isolation and purification of viral nucleic acids from human serum or plasma samples for subsequent *in-vitro* diagnostic purposes. The kit can be used with fresh and frozen human serum and plasma, stabilized with either EDTA or citrate from common blood collection systems. The kit is designed to be used with any downstream application employing enzymatic amplification and detection of RNA and DNA (e.g., RT-PCR, PCR).

The viral nucleic acids isolated and purified with **NucleoSpin® Dx Virus** can be used in qualitative applications (e.g., RT-PCR or PCR for blood screening) as well as in quantitative applications (e.g., detection of viral load by qPCR) employing diagnostic nucleic acid amplification techniques.

Any diagnostic results generated using nucleic acids isolated with the **NucleoSpin® Dx Virus** kit in conjunction with an *in-vitro* diagnostic assay should be interpreted with regard to additional clinical or laboratory findings. To minimize irregularities in diagnostic results, suitable controls for downstream applications (e.g., extraction controls, positive / negative controls) should be used.

The **NucleoSpin® Dx Virus** kit is intended for use by professional users such as technicians and physicians experienced and trained in molecular biological techniques including experience with serum and plasma samples and viral nucleic acid isolation.

The **NucleoSpin® Dx Virus** kit does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in-vitro* diagnostic assay.

Besides human samples also fresh and frozen animal samples can readily be used together with the **NucleoSpin® Dx Virus** kit. Samples include, but are not limited to, serum, plasma, or swabs. It has to be noted that CE IVD labeling of the kit does not apply for animal samples but is limited to human diagnostic use only.

2.2 Product use limitations

The **NucleoSpin® Dx Virus** kit is not for use with human whole blood, tissue, stool samples, or cultured cells.

The kit performance has not been evaluated with other cell-free fluid samples like urine or cerebrospinal fluid.

The kit is also neither specified for the isolation and purification of bacterial, fungal, or parasite nucleic acids from human samples nor for the isolation of viral nucleic acids from human swab samples or other sample collections systems.

2.3 Quality control

In accordance with MACHEREY-NAGEL´s Quality Management System, each lot of **NucleoSpin® Dx Virus** kit is tested against predetermined specifications to ensure consistent product quality.

2.4 Introduction and kit specifications

NucleoSpin® Dx Virus is based on well-established **NucleoSpin®** silica-membrane technology and provides an easy way to isolate viral RNA and viral DNA simultaneously from 150 µL of serum or plasma samples. Purified RNA and DNA are ready-to-use for downstream amplifications like RT-PCR or PCR.

The **NucleoSpin® Dx Virus** procedure is based on a series of simple steps:
First, the serum or plasma samples are lysed in the presence of chaotropic salts. For the purification of viral DNA, Proteinase K is added to the lysis reaction. Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane of the **NucleoSpin® Dx Virus Columns**. Carrier RNA improves binding and recovery of low-concentrated viral RNA and DNA. Contaminations (potential PCR inhibitors) like salts, metabolites, and soluble macromolecular cellular components are removed in washing steps with ethanolic buffers RAW and RAV3. The nucleic acids are finally eluted in 50 µL low salt buffer or water.

The linear range of the **NucleoSpin® Dx Virus** procedure has been determined for HCV RNA and HBV DNA in downstream diagnostic assays (Figures 1 and 2). The kit shows linearity over several orders of magnitude, comprising relevant viral titer for diagnostic purposes.

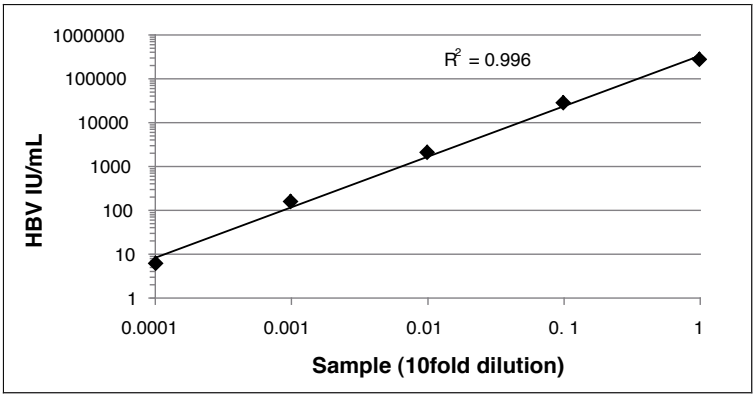

Figure 1: Serial dilution of a plasma sample with high HBV viral load.

Real-time PCR of HBV DNA: Artus RealArt HBV DNA, quantification in Roche LightCycler® 480.

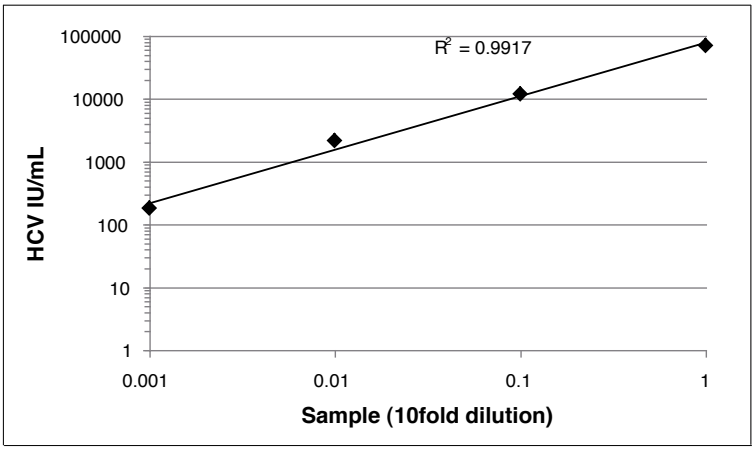

Figure 2: Serial dilution of a plasma sample with high HCV viral load.

Real-time RT-PCR of HCV RNA: Artus RealArt HCV RNA, quantification in Roche LightCycler® 480.

Kit specifications

- **NucleoSpin® Dx Virus** is designed for the rapid preparation of highly pure viral RNA and DNA (e.g., HCV, HIV, HBV, CMV, H1N1) from plasma and serum.
- **NucleoSpin® Dx Virus** is suitable for 150 µL serum or plasma samples.
- The viral nucleic acids isolated and purified with **NucleoSpin® Dx Virus** can be used in qualitative applications (e.g., RT-PCR or PCR for blood screening) as well as in quantitative applications (e.g., detection of viral load by qPCR) employing diagnostic nucleic acid amplification techniques.
- Protocols for isolation of viral RNA, viral DNA, and simultaneous isolation of viral RNA and DNA are included in the user manual.
- The prepared nucleic acids are suitable for applications like automated fluorescent DNA sequencing, RT-PCR, or any kind of enzymatic reaction. The detection limit for certain viruses depends on the individual procedures (e.g., in-house nested (RT-) PCR). To minimize irregularities in diagnostic results, suitable controls for downstream applications (e.g., extraction controls, positive/negative controls) should be used to monitor the purification, amplification, and detection process.
- Besides human samples also fresh and frozen animal samples can readily be used together with the **NucleoSpin® Dx Virus** kit. Samples include, but are not limited to, serum, plasma, or swabs. It has to be noted that CE IVD labeling of the kit does not apply for animal samples but is limited to human diagnostic use only.

Table 1: Kit specifications at a glance	
Parameter	**NucleoSpin® Dx Virus**
Technology	Silica-membrane technology
Sample material	Serum or plasma
Sample volume	150 µL
Elution volume	50 µL
Preparation time	30 min / 4–6 preps
Processing	Centrifugation

2.5 Remarks regarding sample quality and preparation

- **NucleoSpin® Dx Virus** is suitable for human serum or plasma samples. For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and non-viscous sample lysate before adjusting binding conditions and loading the sample onto the **NucleoSpin® Dx Virus Column**. Check all samples (especially old or frozen samples) for precipitates.
- It is very important to avoid clearing samples by centrifugation / filtration before the RAV1-lysis step, because viruses may be associated with particles or aggregates. Incubation with Buffer RAV1 can be prolonged to dissolve and digest residual cell structures, precipitates and virus particles. However, RNA is sensitive and prolonged incubation may cause decreased yields.

2.6 Remarks regarding elution

- Pure nucleic acids are finally eluted under low ionic strength conditions with RNase-free H_2O (pH about 7–8) or slightly alkaline Buffer RE (5 mM Tris-HCl, pH 8.5), both are supplied with **NucleoSpin® Dx Virus**.
- RNA should be eluted with the RNase-free H_2O and DNA with Elution Buffer RE.
- To elute both types of nucleic acids together, use RNase-free H_2O provided with the kit, preheated to 70 °C.

3 Storage conditions and preparation of working solutions

Attention: *Buffer RAV1 contains guanidinium thiocyanate and Buffer RAW contains guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.*

- Check all components for damages after receiving the kit. If kit contents like buffer bottles or blister packages are damaged, contact MACHEREY-NAGEL technical support and customer service, or your local distributor.
- Do not use damaged kit components.
- Upon arrival the **NucleoSpin® Dx Virus** kit should be stored at room temperature (18–25 °C). It is NOT required to open the kit on delivery and remove individual components for separate storage.
- **NucleoSpin® Dx Virus Columns** can be used until the expiration date on the kit box.
- Use RNase-free equipment.

Before starting the **NucleoSpin® Dx Virus** protocol prepare the following:

- **Lyophilized Proteinase K** can be stored at room temperature (18–25 °C) until the expiration date without decrease in performance. Before first use of the kit, add the indicated volume of **Proteinase Buffer PB** to dissolve lyophilized Proteinase K. Reconstituted Proteinase K should be stored at -20 °C for up to 6 months, but only until the expiration date.
- **Carrier RNA:** Before first use, add **1 mL Lysis Buffer RAV1** to the Carrier RNA vial. Dissolve the Carrier RNA and pipette the solution back to the RAV1 bottle.

  *Note: Due to the production procedure and the small amount of **Carrier RNA** contained in the vial, the Carrier RNA may hardly be visible.*

  Lysis Buffer RAV1 including Carrier RNA can be stored at 4 °C for up to 4 weeks. Storage at 4 °C or below may cause salt precipitation. If precipitates are visible, make sure to dissolve all precipitates before use by heating at 40–60 °C for a maximum of 5 min. Carrier RNA dissolved in Buffer RAV1 and stored at -20 °C is stable for at least one year.

  Do not warm up Buffer RAV1 containing Carrier RNA more than 4 times! Frequent warming, temperatures > 80 °C, and extended heat incubation will accelerate the degradation of Carrier RNA.

- **Wash Buffer RAV3:** Add the indicated volume (see table below or on the bottle) of ethanol (96–100 %) to **Wash Buffer RAV3 Concentrate**. Mark the label of the bottle to indicate that the ethanol is added. Store Wash Buffer RAV3 at room temperature. Wash Buffer RAV3 can be stored at room temperature (18–25 °C) for up to one year but only until the expiration date.

	NucleoSpin® Dx Virus
	50 preps
REF	**740895.50**
Wash Buffer RAV3 (Concentrate)	12 mL Add 48 mL ethanol
Proteinase K	30 mg Add 1.35 mL Proteinase Buffer PB

4 Safety instructions

The following components of the **NucleoSpin® Dx Virus** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

Component *Inhalt*	Hazard contents *Gefahrstoff*	GHS symbol *GHS Symbol*	Hazard phrases *H-Sätze*	Precaution phrases *P-Sätze*
RAV1	Guanidinium thiocyanate 30–60 % *Guanidiniumthiocyanat 30–60 %*	Warning *Achtung*	302, 412, EUH031	260, 273, 301+312, 330
RAW	Guanidine hydrochloride 24–36 % + ethanol 35-55 % *Guanidinhydrochlorid 24–36 % + Ethanol 35–55 %*	Warning *Achtung*	226, 302	210, 233, 301+312, 330, 403+235
Proteinase K	Proteinase K, lyophilized *Proteinase K, lyophilisiert*	Danger *Gefahr*	315, 317, 319, 334, 335	261, 280, 302+352, 304+340, 305+351+338, 312, 332+313, 337+313, 342+311, 363, 403+233

Hazard phrases

H 226	Flammable liquid and vapour. *Flüssigkeit und Dampf entzündbar.*
H 302	Harmful if swallowed. *Gesundheitsschädlich bei Verschlucken.*
H 315	Causes skin irritation. *Verursacht Hautreizungen.*
H 317	May cause an allergic skin reaction. *Kann allergische Hautreaktionen verursachen.*
H 319	Causes serious eye irritation. *Verursacht schwere Augenreizung.*
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled. *Kann bei Einatmen Allergie, asthmaartige Symptome oder Atembeschwerden verursachen.*
H 335	May cause respiratory irritation. *Kann die Atemwege reizen.*
H 412	Harmful to aquatic life with long lasting effects. *Schädlich für Wasserorganismen, mit langfristiger Wirkung.*

EUH031	Contact with acids liberates toxic gas. *Entwickelt bei Berührung mit Säure giftige Gase.*

Precaution phrases

P 210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. *Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.*
P 233	Keep container tightly closed *Behälter dicht verschlossen halten.*
P 260	Do not breathe vapours. *Dampf nicht einatmen.*
P 261	Avoid breathing dust. *Einatmen von Staub vermeiden.*
P 273	Avoid release to the environment. *Freisetzung in die Umwelt vermeiden.*
P 280	Wear protective gloves / eye protection. *Schutzhandschuhe / Augenschutz tragen.*
P 301+312	IF SWALLOWED: Call a POISON CENTER/ doctor/…/if you feel unwell. *BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /… anrufen.*
P 302+352	IF ON SKIN: Wash with plenty of water/… *BEI KONTAKT MIT DER HAUT: Mit viel Wasser/… waschen.*
P 304+340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. *BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.*
P 305+351+338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. *BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.*
P 312	Call a POISON CENTER/ doctor/…/if you feel unwell. *Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt /… anrufen.*
P 330	Rinse mouth. *Mund ausspülen.*
P 332+313	If skin irritation occurs: Get medical advice / attention. *Bei Hautreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 337+313	Get medical advice / attention. *Bei anhaltende Augenreizung: Ärztlichen Rat einholen / ärztliche Hilfe hinzuziehen.*
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER/ doctor/… *Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM /Arzt/… anrufen.*
P 363	Wash contaminated clothing before reuse. *Kontaminierte Kleidung vor erneutem Tragen waschen.*
P 403+233	Store in a well ventilated place. Keep container tightly closed. *Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.*
P 403+235	Store in a well ventilated place. Keep cool. *Kühl an einem gut belüfteten Ort aufbewahren.*

For further information please see Material Safety Data Sheets ***(www.mn-net.com).***
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern ***(www.mn-net.com)****.*

When working with the **NucleoSpin® Dx Virus** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at *http://www.mn-net.com/msds*).

Caution: Guanidinium thiocyanate in Lysis Buffer RAV1 and guanidine hydrochloride in Wash Buffer RAW can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste.

The waste generated with the **NucleoSpin® Dx Virus** kit has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

5 Viral nucleic acid purification with NucleoSpin® Dx Virus

The procedures below provide instructions for processing a single plasma or serum sample. However, several samples can be processed at the same time; the number depends on the capacity of the microcentrifuge used.

Before starting the preparation:

- Check that Wash Buffer RAV3 and Proteinase K were prepared according to section 3.
- Check that Carrier RNA has been dissolved in Lysis Buffer RAV1 according to section 3.
- Check that 96–100 % ethanol (denatured or non-denatured) is available to adjust nucleic acid binding conditions.
- Set an incubator (e.g., heating block) or water bath to 70 °C.
- Equilibrate the plasma/serum samples to room temperature (18–25 °C). Make sure that the samples are mixed well.
- If a precipitate has formed in Lysis Buffer RAV1 or Buffer RAW, incubate the buffer at 40–60 °C until the precipitate is dissolved.
- Generally, do not mix reagents and columns from different kits and lots.
- Heat RNase-free H_2O / Elution Buffer RE to 70 °C for final elution of nucleic acids.
- Do not add Proteinase K solution directly to Lysis Buffer RAV1. The sample has to be combined with the Lysis Buffer RAV1 before addition of Proteinase K.
- All centrifugation steps should be carried out at room temperature (18–25 °C).

5.1 Protocol-at-a-glance

Supplemental protocol-overview:

Carefully read the detailed protocol (section 5.2–5.4) before starting the procedure.
Note: **The protocols differ in Proteinase K lysis step (step 3) and elution step (step 24) only.**

		Viral **RNA** isolation procedure (section 5.2)	Viral **DNA** isolation procedure (section 5.3)	Viral **RNA + DNA** isolation procedure (section 5.4)
Provide sample, lyse viruses, clear lysate	**1**	**150 µL sample** in Lysis Tubes	**150 µL sample** in Lysis Tubes	**150 µL sample** in Lysis Tubes
	2	**600 µL Buffer RAV1** containing Carrier RNA	**600 µL Buffer RAV1** containing Carrier RNA	**600 µL Buffer RAV1** containing Carrier RNA
	3	*Note: No Proteinase K is used for the isolation of viral RNA only*	**20 µL Proteinase K** *(Incubate at least 1 min at room temperature)*	**20 µL Proteinase K** *(Incubate at least 1 min at room temperature)*
	4	Pipette mixture up and down and vortex well	Pipette mixture up and down and vortex well	Pipette mixture up and down and vortex well
	5	Incubate at **70 °C** for **5 min**	Incubate at **70 °C** for **5 min**	Incubate at **70 °C** for **5 min**
	6	Short spin to clean the lid	Short spin to clean the lid	Short spin to clean the lid
Adjust binding conditions	**7**	**600 µL ethanol**	**600 µL ethanol**	**600 µL ethanol**
	8	Mix by vortexing (10–15 s)	Mix by vortexing (10–15 s)	Mix by vortexing (10–15 s)
Bind RNA/ DNA	**9**	Load **700 µL lysate** onto the NucleoSpin® Dx Virus Column	Load **700 µL lysate** onto the NucleoSpin® Dx Virus Column	Load **700 µL lysate** onto the NucleoSpin® Dx Virus Column
	10	8,000 x *g*, 1 min	8,000 x *g*, 1 min	8,000 x *g*, 1 min

	11	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube
	12	Load the **residual lysate** (ca. 650 µL) onto the column	Load the **residual lysate** (ca. 650 µL) onto the column	Load the **residual lysate** (ca. 650 µL) onto the column
	13	8,000 x *g*, 1 min	8,000 x *g*, 1 min	8,000 x *g*, 1 min
	14	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube
Wash silica membrane	**15**	**500 µL RAW**	**500 µL RAW**	**500 µL RAW**
	16	8,000 x *g*, 1 min	8,000 x *g*, 1 min	8,000 x *g*, 1 min
	17	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube
	18	**600 µL RAV3**	**600 µL RAV3**	**600 µL RAV3**
	19	8,000 x *g*, 1 min	8,000 x *g*, 1 min	8,000 x *g*, 1 min
	20	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube	Transfer the NucleoSpin® Dx Virus Column to a new Collection Tube
	21	**200 µL RAV3**	**200 µL RAV3**	**200 µL RAV3**
	22	11,000 x *g*, 3 min	11,000 x *g*, 3 min	11,000 x *g*, 3 min
Elute RNA/ DNA	**23**	Transfer the NucleoSpin® Dx Virus Column to an Elution Tube	Transfer the NucleoSpin® Dx Virus Column to an Elution Tube	Transfer the NucleoSpin® Dx Virus Column to an Elution Tube
	24	**50 µL RNase-free H_2O** (70 °C); Incubate 1–2 min	**50 µL Buffer RE** (70 °C); Incubate 1–2 min	**50 µL RNase-free H_2O** (70 °C); Incubate 1–2 min
	25	11,000 x *g*, 1 min	11,000 x *g*, 1 min	11,000 x *g*, 1 min

5.2 Viral RNA isolation procedure

1. Provide **150 µL sample** in a Lysis Tube (1.5 mL, provided).
2. Add **600 µL Buffer RAV1** containing Carrier RNA to the Lysis Tube.
3. *Note: No Proteinase K is used for the isolation of viral RNA only.*
4. Pipette mixture up and down and vortex well.
5. Incubate for **5 min** at **70 °C**.
6. **Briefly centrifuge** Lysis Tube (approx. 1 s at 2,000 x *g*) to remove drops from the lid (short spin only).

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7. Add **600 µL ethanol** (96–100 %) to the clear lysate.
8. Mix by vortexing (10–15 s).

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9. Carefully load **700 µL of the lysate** onto the **NucleoSpin® Dx Virus Column** placed in a Collection Tube and close the lid.
10. **Centrifuge 1 min** at **8,000 x *g***.
11. Place the **NucleoSpin® Dx Virus Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.
12. Load the **residual lysate** (approx. 650 µL) onto the NucleoSpin® Dx Virus Column and close the lid.
13. **Centrifuge 1 min** at **8,000 x *g***.
14. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

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15. Add **500 µL Buffer RAW** to the NucleoSpin® Dx Virus Column.
16. **Centrifuge 1 min** at **8,000 x *g***.
17. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.
18. Add **600 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.
19. **Centrifuge 1 min** at **8,000 x *g***.
20. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

21. Add **200 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.
22. **Centrifuge 3 min** at **11,000 x *g***.
23. Place the NucleoSpin® Dx Virus Column into an Elution Tube (1.5 mL, provided) and discard the Collection Tube with flow-through from the previous step.
24. Add **50 µL RNase-free H_2O** (preheated to 70 °C) and incubate for 1–2 min.
25. **Centrifuge 1 min** at **11,000 x *g*** to elute nucleic acid from the column.

5.3 Viral DNA isolation procedure

1. Provide **150 µL sample** in a Lysis Tube (1.5 mL, provided).
2. Add **600 µL Buffer RAV1** containing Carrier RNA to the Lysis Tube.
3. Add **20 µL Proteinase K** solution to the Lysis Tube.
   Note: Proteinase K is necesary for lysis of DNA viruses.
4. Pipette mixture up and down and vortex well.
   Note: Make sure that the mixture incubates at least 1 min at room temperature before starting the heat incubation.
5. Incubate for **5 min** at **70 °C**.
6. **Briefly centrifuge** Lysis Tube (approx. 1 s at 2,000 x *g*) to remove drops from the lid (short spin only).

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7. Add **600 µL ethanol** (96–100 %) to the clear lysate.
8. Mix by vortexing (10–15 s).

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9. Carefully load **700 µL of the lysate** onto the **NucleoSpin® Dx Virus Column** placed in a Collection Tube and close the lid.
10. **Centrifuge 1 min** at **8,000 x *g***.
11. Place the **NucleoSpin® Dx Virus Column** into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.
12. Load the **residual lysate** (approx. 650 µL) onto the NucleoSpin® Dx Virus Column and close the lid.
13. **Centrifuge 1 min** at **8,000 x *g***.
14. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

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15. Add **500 µL Buffer RAW** to the NucleoSpin® Dx Virus Column.
16. **Centrifuge 1 min** at **8,000 x *g***.
17. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.
18. Add **600 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.
19. **Centrifuge 1 min** at **8,000 x *g***.

20. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

21. Add **200 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.

22. **Centrifuge 3 min** at **11,000 x *g***.

23. Place the NucleoSpin® Dx Virus Column into an Elution Tube (1.5 mL, provided) and discard the Collection Tube with flow-through from the previous step.

24. Add **50 µL Buffer RE** (preheated to 70 °C) and incubate for 1–2 min.

25. **Centrifuge 1 min** at **11,000 x *g*** to elute nucleic acid from the column.

5.4 Simultaneous viral RNA and DNA isolation procedure

1. Provide **150 µL sample** in a Lysis Tube (1.5 mL, provided).
2. Add **600 µL Buffer RAV1** containing Carrier RNA to the Lysis Tube.
3. Add **20 µL Proteinase K** solution to the Lysis Tube.
   Note: Proteinase K is necesary for lysis of DNA viruses.
4. Pipette mixture up and down and vortex well.
   Note: Make sure that the mixture incubates at least 1 min at room temperature before starting the heat incubation.
5. Incubate for **5 min** at **70 °C**.
6. **Briefly centrifuge** Lysis Tube (approx. 1 s at 2,000 x *g*) to remove drops from the lid (short spin only).

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7. Add **600 µL ethanol** (96–100 %) to the clear lysate.
8. Mix by vortexing (10–15 s).

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9. Carefully load **700 µL of the lysate** onto the **NucleoSpin® Dx Virus Column** placed in a Collection Tube and close the lid.
10. **Centrifuge 1 min** at **8,000 x *g***.
11. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.
12. Load the **residual lysate** (approx. 650 µL) onto the NucleoSpin® Dx Virus Column and close the lid.
13. **Centrifuge 1 min** at **8,000 x *g***.
14. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.

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15. Add **500 µL Buffer RAW** to the NucleoSpin® Dx Virus Column.
16. **Centrifuge 1 min** at **8,000 x *g***.
17. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.
18. Add **600 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.
19. **Centrifuge 1 min** at **8,000 x *g***.

20. Place the NucleoSpin® Dx Virus Column into a new Collection Tube (2 mL, provided) and discard the Collection Tube with flow-through from the previous step.
21. Add **200 µL Buffer RAV3** to the NucleoSpin® Dx Virus Column.
22. **Centrifuge 3 min** at **11,000 x *g***.
23. Place the NucleoSpin® Dx Virus Column into an Elution Tube (1.5 mL, provided) and discard the Collection Tube with flow-through from the previous step.
24. Add **50 µL RNase-free H_2O** (preheated to 70 °C) and incubate for 1–2 min.
25. **Centrifuge 1 min** at **11,000 x *g*** to elute nucleic acid from the column.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Small amounts or no viral nucleic acids in the eluate	*Low viral load in the sample* • The nucleic acid yield depends on the viral load in the sample. *Problems with Carrier RNA* • Carrier RNA not added. • See remarks concerning storage of Buffer RAV1 with Carrier RNA (section 3). *Proteinase K digestion may be necessary* • Choose the appropriate protocol for viral RNA or viral DNA isolation, see section 5.1. *Viral nucleic acids degraded* • Samples should be processed immediately. Ensure appropriate storage conditions up to the processing. • Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Buffer RAV1, Carrier RNA and Elution Buffer RE.
Problems with subsequent detection	*Reduced sensitivity* • Change the volume of eluate added to the PCR/RT-PCR. *Ethanol carry-over* • Prolong centrifugation step (step 22) in order to remove Buffer RAV3 completely.

6.2 Ordering information

Product	REF	Pack of
CE-IVD marked kits		
NucleoSpin® Dx Virus	740895.50	50
NucleoSpin® Dx Blood	740899.50/.250	50/250
Kits for research purposes		
NucleoSpin® Virus	740983.10/.50/.250	10/50/250
NucleoSpin® RNA Virus F	740958	25
NucleoSpin® totalRNA FFPE XS	740969.10/.50/.250	10/50/250
NucleoSpin® totalRNA FFPE	740982.10/.50/.250	10/50/250
NucleoSpin® DNA FFPE XS	740980.10/.50/.250	10/50/250
NucleoSpin® Blood	740951.10/.50/.250	10/50/250
NucleoSpin® Tissue	740952.10/.50/.250	10/50/250
NucleoSpin® Tissue XS	740901.10/.50/.250	10/50/250
NucleoSpin® miRNA	740971.10/.50/.250	10/50/250
Proteinase K	740506	100 mg
Collection Tubes (2 mL)	740600	1000

Visit ***www.mn-net.com*** for more detailed product information.

6.3 Product use restriction/warranty

The **NucleoSpin® Dx Virus** kit is a generic system for the isolation and purification of viral nucleic acids from human plasma or serum samples for subsequent *in-vitro* diagnostic purposes.

The kit is designed to be used with any downstream application employing enzymatic amplification and detection of RNA and DNA (e.g., RT-PCR, PCR).

Any and all diagnostic results generated using nucleic acids isolated with the **NucleoSpin® Dx Virus** kit in conjunction with a diagnostic assay should be interpreted with regard to additional clinical or laboratory findings.

The **NucleoSpin® Dx Virus** kit does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in-vitro* diagnostic assay. ONLY MACHEREY-NAGEL products specially labeled as IVD are suitable for *IN-VITRO*-diagnostic use.

The **NucleoSpin® Dx Virus** kit is intended for use by professional users such as technicians and physicians experienced and trained in molecular biological techniques including experience with serum and plasma samples and viral nucleic acid isolation.

For safety instructions please refer to the respective chapter in the user manual. **NucleoSpin® Dx Virus** kit shall exclusively be used in an adequate test environment, i.e. a suitable laboratory setting. The respective user is liable for any and all damages resulting from application of the **NucleoSpin® Dx Virus** kit for use deviating from the intended use as specified in the user manual.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL´s sole obligation and the customer´s sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

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Last updated: 07/2010, Rev. 03

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